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Effect of Interferon on a Nonenveloped DNA Virus (TT Virus) Associated With Acute and Chronic Hepatitis of Unknown Etiology

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An unenveloped DNA virus named TT virus (TTV) has been reported in association with acute and chronic hepatitis of unknown etiology. The effect of interferon on TTV was evaluated in the patients with chronic hepatitis C who were coinfected with TTV. TTV DNA was determined by a polymerase chain reaction with heminested primers in the 96 patients with chronic hepatitis C who received interferon-α (516 million units in 26 weeks) and followed for 24 months thereafter. TTV DNA was detected in 31 (32%) patients before therapy. TTV DNA became undetectable during interferon therapy and remained absent in 14 (45% of the 31 patients) through 24 months thereafter. The four patients with pretreatment TTV DNA titer $\geq 10^3$/ml did not respond. These results indicate that TTV is sensitive to interferon, and the response would be inversely correlated with pretreatment viral titers. ***J. Med. Virol. 58:196–200, 1999.***

KEY WORDS: hepatitis viruses; hepatitis C virus; chronic hepatitis; interferons

INTRODUCTION

Posttransfusion hepatitis occurs in recipients of blood units screened for hepatitis B virus (HBV) and hepatitis C virus (HCV) [Alter and Bradley, 1995]. A significant proportion of patients with chronic hepatitis are without markers of ongoing infection with recognized hepatitis viruses, and the cause remains unknown [Kodali et al., 1994]. Furthermore, fulminant hepatitis in most cases is not attributable to known hepatitis viruses [Wright, 1993]. This background points to the presence of yet unidentified hepatitis virus(es).

In an attempt to account for hepatitis in the patients without markers of known hepatitis viruses, GB virus C (GBV-C) and hepatitis G virus (HGV) were reported successively by two independent groups of investigators [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. Since these viruses represent separate isolates of the same virus [Zuckerman, 1995], they will be referred to collectively as GBV-C/HGV. Later studies, however, have not demonstrated a significant hepatitis-inducing capacity of GBV-C/HGV [Alter, 1997; Miyakawa and Mayumi, 1997], or its replication in the liver [Pessoa et al., 1998]. Hence there would still be virus(es) responsible for hepatitis of unknown etiology.

A nonenveloped DNA virus named TT virus (TTV) has been recovered from serum of a patient with posttransfusion hepatitis of unknown etiology [Nishizawa et al., 1997]. DNA of TTV was detected by a polymerase chain reaction (PCR) in titers in parallel with alanine aminotransferase (ALT) levels in three of the five patients with posttransfusion hepatitis of unknown etiology in Japan [Nishizawa et al., 1997]. Posttransfusion hepatitis of unknown etiology occurred significantly more frequently in the patients who acquired TTV viremia after transfusion than in those who did not (4 of 18 [22%] vs 0 of 19 [0%], $P < .05$) [Fujiwara et al., 1998]. TTV DNA is detected frequently in the patients with fulminant hepatitis (9 of 19 [47%]) and in those with chronic liver disease (41 of 90 [46%]) of unknown etiology in Japan [Okamoto et al., 1998a]. In the United States, it is reported in 1% (1 of 100) of blood donors, as well as in 15% (5 of 33) of patients with cryptogenic cirrhosis and 27% (3 of 11) of patients with idiopathic fulminant hepatic failure [Charlton et al., 1998].

TTV is transmitted parenterally, because it is highly prevalent in the patients with hemophilia (19 of 28 [68%]), those on maintenance hemodialysis (26 of 57 [46%]) and intravenous drug users (14 of 35 [40%]) [Okamoto et al., 1998a]. TTV may be transmitted non-

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parenterally, via a fecal–oral route, since it is secreted into feces [Okamoto et al., 1998b]. Dual modes of transmission may be responsible for the high prevalence of TTV DNA in healthy blood donors (34 of 290 [12%]) in Japan. Recently, TTV has been reported in 19 of 1,000 nonrenumerated blood donors [Simmonds et al., 1998] and in 18 of 72 patients (25%) with liver disease [Naoumov et al., 1998] in England. It is frequent, also, in blood donors and patients with chronic liver disease in Thailand [Tanaka et al., 1998]. Hence TTV would be prevalent worldwide.

In an attempt to evaluate the response of TTV to interferon (IFN), 96 patients with chronic hepatitis C who had received IFN were tested for TTV DNA. The 31 patients whose test results were positive for TTV DNA were followed up during IFN therapy and for 24 months thereafter.

MATERIALS AND METHODS

Patients

A total of 96 patients with chronic hepatitis C received IFN therapy and were followed up for 24 months thereafter during June 1992 through June 1994. They were 46 ± 9 (mean ± SD) years old (range: 24–66 years), and included 74 males and 22 females. Diagnosis of liver disease was chronic persistent hepatitis (CPH) in 25 patients, chronic active hepatitis 2A (CAH2A) in 43, chronic active hepatitis 2B (CAH2B) in 19, and liver cirrhosis (LC) in 9. The diagnosis was made histologically by the criteria of De Groote et al. [1968], and the histological activity index score was calculated by the method of Knodell et al. [1981]. None of these patients was coinfected with HBV or human immunodeficiency virus type 1.

Sera were taken at admission, just before the start of IFN therapy, during IFN (2 and 12 weeks), at the completion (26 weeks), and after IFN (3, 6, 12, and 24 months). The results of HCV RNA and GBV-C/HGV RNA for the 96 patients, in response to IFN, have been reported previously [Inoue et al., 1997]. TTV DNA was determined on the stored sera. The study was approved by the Ethics Committee of the hospital, and informed consent was obtained from every patient.

Interferon Therapy

Human lymphoblastoid IFN (IFN-α) (Sumiferon, Sumitomo Pharmaceutical Co., Osaka, Japan) was given intramuscularly at a dose of 6 million units (MU) daily for 2 weeks and then three times a week for 24 weeks, with a total dose of 516 MU.

Determination of TTV DNA

Serum (100 μl) was pretreated with proteinase K and sodium dodecyl sulfate, and nucleic acids were extracted with phenol and chloroform by the method reported previously [Okamoto et al., 1990]. Extracted nucleic acids were dissolved in 10 μl of Tris-HCl buffer (10 mM, pH 8.0) supplemented with 1 mM EDTA (TE buffer), heated at 95°C for 15 minutes and quickly chilled on ice. They were tested for TTV DNA by PCR with heminested primers, NG059 and NG063 in the first round and NG061 and NG063 in the second round [Okamoto et al., 1998a], and Perkin-Elmer AmpliTaq DNA Polymerase (Roche Molecular Systems, New Jersey, USA) by the method described elsewhere [Okamoto et al., 1998b]. A relative TTV DNA titer was determined by the limiting dilution method and expressed as DNA copies (10^N) per 1 ml of serum.

TABLE I. Comparison of the Patients With Chronic Hepatitis C Who Were and Who Were Not Coinfected With TTV

Features[a]	TTV DNA (+) (n = 31)	TTV DNA (–) (n = 65)	Differences
Age (years)	47 ± 7	46 ± 10	NS
Male	25 (81%)	49 (75%)	NS
Risk factors			
Transfusion	12 (39%)	14 (22%)	NS
Acute hepatitis	4 (12%)	11 (17%)	NS
IV drug/tattooing	2 (6%)	7 (11%)	NS
Liver histopathology			
CPH	8 (26%)	17 (26%)	NS
CAH2A	16 (52%)	27 (42%)	NS
CAH2B	4 (13%)	15 (23%)	NS
LC	3 (10%)	6 (9%)	NS
Knodell's histology score	8.9 ± 3.7	9.4 ± 3.7	NS
HCV			
RNA titer ($\geq 10^4$/ml)	22 (71%)	33 (51%)	NS
Genotype (1b)	22 (71%)	40 (62%)	NS
Response to IFN	8 (26%)	25 (38%)	NS
GBV-C/HGV RNA	5 (16%)	5 (8%)	NS
ALT (U/l)	110 ± 75	111 ± 77	NS
AST (U/l)	68 ± 49	61 ± 33	NS
ALP (U/l)	163 ± 60	169 ± 56	NS
γ-GTP (U/l)	67 ± 73	76 ± 82	NS
Chol (mg/100 ml)	174 ± 32	159 ± 27	$P < .05$
Hemoglobin (g/100 ml)	14.8 ± 1.2	14.7 ± 1.6	NS
RBC ($\times 10^4$/μl)	462 ± 41	459 ± 44	NS
WBC ($\times 10^2$/μl)	55.8 ± 14.6	56.3 ± 16.4	NS
Platelets ($\times 10^4$/μl)	19.5 ± 6.2	19.9 ± 6.0	NS

[a]*Abbreviations* (*normal values*): ALT, alanine aminotransferase (2–31 U/l): AST, aspartate aminotransferase (10–31 U/l); ALP, alkaline phosphatase (92–320 U/l); γ-GTP, γ-glutamyl transpeptidase (6–94 U/l); Chol, total cholesterol (126–251 mg/100 ml); hemoglobin (11.4–17.1 g/100 ml); RBC, red blood cells (368–556 × 10^4/μl); WBC, white blood cells (37–100 × 10^2/μl); platelets (16–34 × 10^4/μl); NS, not significant.

Statistical Analyses

Frequency between groups was compared using the χ^2 test or Fisher's exact test, and group means were compared using Student's t test.

RESULTS

TTV DNA in the Patients With Chronic Hepatitis C

TTV DNA was detected in pretreatment sera from 31 of the 96 (32%) patients with chronic hepatitis C. Table I compares various features between the 31 patients with TTV DNA and the remaining 65 patients without TTV DNA. A history of transfusion tended to be more frequent in the patients with TTV DNA than in those

TABLE II. Response of TTV to Interferon in the 31 Patients With Chronic Hepatitis C

Case no.	Age and sex	Diagnosis	Response to IFN: HCV RNA	Response to IFN: GBV-C/HGV RNA	Effect of IFN on TTV DNA (relative titer: 10^N/ml): Pre	During: 2W	During: 12W	During: End	After IFN: 3 M	After IFN: 6 M	After IFN: 12 M	After IFN: 24 M
Group A: Responders With Respect to TTV												
1	57M	CAH2B	NR	—	1	—	—	—	—	—	—	—
2	55M	CAH2A	R	—	1	—	—	—	—	—	—	—
3	52M	CAH2A	NR	—	1	—	—	—	—	—	—	—
4	48M	CAH2A	R	—	1	1	—	—	—	—	—	—
5	39M	CPH	R	—	1	1	—	—	—	—	—	—
6	44M	CAH2A	NR	—	2	—	—	—	—	—	—	—
7	49M	CAH2A	NR	—	2	—	—	—	—	—	—	—
8	48M	CAH2A	R	—	2	—	—	—	—	—	—	—
9	50F	CAH2B	NR	—	2	—	—	—	—	—	—	—
10	50M	LC	NR	—	2	—	—	—	—	—	—	—
11	24M	CAH2A	NR	—	2	1	—	—	—	—	—	—
12	42M	CAH2A	NR	—	2	1	—	—	—	—	—	—
13	55M	CPH	NR	—	2	1	—	—	—	—	—	—
14	49M	CAH2A	NR	—	2	1	—	—	—	—	—	—
Group B: Nonresponders With Respect to TTV												
15	45F	LC	NR	—	1	—	—	—	1	1	1	1
16	54F	CAH2A	NR	—	1	1	—	—	1	1	1	1
17	40M	CAH2B	NR	—	1	—	—	1	2	1	1	1
18	42F	CPH	NR	—	2	—	—	—	1	1	1	1
19	39M	CAH2B	NR	—	2	—	—	—	—	2	2	2
20	36M	CAH2A	NR	—	2	—	—	—	3	2	2	3
21	48M	CPH	NR	—	2	1	—	—	1	1	1	1
22	54F	CAH2A	NR	—	2	1	—	—	1	1	—	1
23	51M	CAH2A	NR	NR	2	1	—	—	1	2	1	1
24	55M	CPH	NR	NR	2	1	—	—	1	1	1	1
25	46M	CPH	NR	—	2	1	—	—	2	2	1	1
26	53F	CAH2A	R	—	2	1	1	—	1	2	1	2
27	51M	CPH	R	—	2	2	—	—	—	2	2	2
28	49M	CAH2A	R	—	3	1	—	—	2	1	1	1
29	37M	CAH2A	NR	R	3	2	—	—	3	2	2	2
30	54M	LC	NR	NR	3	2	—	—	2	2	1	2
31	42M	CPH	R	NR	4	—	—	—	3	2	2	1

R, responders; NR, nonresponders; W, weeks; M, months.

without (39% vs 22%). GBV-C/HGV RNA was more frequent in the patients with TTV DNA than in those without (16% vs 8%). Of the 96 patients, 60 (63%) were infected with HCV alone, 26 (27%) were coinfected with HCV and TTV, and 5 (5%) were coinfected with HCV and GBV-C/HGV; a triple infection with HCV, TTV, and GBV-C/HGV occurred in 5 (5%) patients.

There were no differences in the histopathology of the liver or the Knodell's histological activity index score between the patients with TTV DNA and those without it. The response to IFN with respect to HCV was a little less frequent in the patients with TTV DNA than in those without (26% vs 38%), which might be attributed to the more common high HCV RNA titers (71% vs 51%) and genotype 1b (71% vs 62%) in the patients coinfected with TTV.

Response to IFN of HCV, GBV-C/HGV, and TTV in the 31 Patients Who Were Coinfected

Table II gives relative titers of TTV DNA in the 31 patients followed before, during, at the completion, and after IFN therapy. The response to IFN is shown, also, with respect to HCV in all of the patients, and to GBV-C/HGV in the five coinfected patients. Response to IFN was defined by the clearance of viral nucleic acids during IFN, which persisted until 6 months after the therapy. All the patients who lost viral nucleic acids from serum 6 months after the completion of IFN stayed negative for it throughout the observation until 24 months after IFN, except for one patient with GBV-C/HGV in whom GBV-C/HGV RNA reappeared at 24 months after the therapy.

Of the 31 patients, the response with respect to HCV was observed in 8 (26%) patients, while that with respect to TTV was achieved in 14 (45%) patients. Of the 14 responders with respect to TTV, 4 (29%) responded also with respect to HCV; none was positive for GBV-C/HGV RNA. By contrast, of the 17 nonresponders to TTV, 5 (29%) had a triple infection with TTV, HCV, and GBV-C/HGV; only one each of these five patients responded in respect to HCV or GBV-C/HGV. In addition, 4 of the 17 (24%) nonresponders with respect to TTV responded with respect to HCV. Of these, two were infected with HCV of genotype 2a and one with

TABLE III. Comparison of the Patients With Chronic Hepatitis C Coinfected With TTV Who Cleared and Who Did Not Clear TTV DNA After IFN Therapy

Features	Responders (n = 14)	Nonresponders (n = 17)	Differences
Age (years)	47 ± 8	47 ± 6	NS
Male	13 (93%)	12 (71%)	NS
Risk factors			
Transfusion	7 (50%)	5 (29%)	NS
Acute hepatitis	2 (14%)	2 (12%)	NS
IV drug/tattooing	0	2 (12%)	NS
Liver histopathology			
CPH	2 (14%)	6 (35%)	NS
CAH2A	9 (64%)	7 (41%)	NS
CAH2B	2 (14%)	2 (12%)	NS
LC	1 (7%)	2 (12%)	NS
Knodell's histology score	9.1 ± 3.1	8.8 ± 4.2	NS
TTV DNA titer ($\geq 10^3$/ml)	0	4 (24%)	NS
HCV			
RNA titer ($\geq 10^4$/ml)	8 (57%)	14 (82%)	NS
Genotype (1b)	10 (71%)	12 (71%)	NS
Response to IFN	4 (29%)	4 (24%)	NS
GBV-C/HGV RNA	0	5 (29%)	$P < .05$
ALT (U/l)	114 ± 92	107 ± 60	NS
AST (U/l)	65 ± 52	70 ± 48	NS
ALP (U/l)	162 ± 63	163 ± 59	NS
γ-GTP (U/l)	51 ± 51	79 ± 86	NS
Chol (mg/100 ml)	171 ± 29	177 ± 36	NS
Hemoglobin (g/100 ml)	15.2 ± 0.7	14.4 ± 1.5	NS
RBC ($\times 10^4/\mu$l)	477 ± 32	449 ± 45	NS
WBC ($\times 10^2/\mu$l)	61.4 ± 14.5	51.2 ± 13.4	NS
Platelets ($\times 10^4/\mu$l)	21.8 ± 7.7	17.5 ± 3.8	NS

See Table 1 for abbreviations.

that of 2b; the remaining patient was infected with HCV of genotype 1b at a low titer (10^1/ml).

Levels of TTV DNA During and After IFN

During IFN, TTV DNA decreased in titer or was cleared from the serum in all the 31 patients coinfected with HCV and TTV. By 2 weeks after the start of IFN, 14 (45%) lost TTV DNA from the serum, and only 1 (3%) remained positive for TTV DNA at 12 weeks; she lost TTV DNA on the completion of IFN, however (Case 26). TTV DNA reappeared in serum during IFN in one patient (Case 17). Of the 16 patients who lost TTV DNA at 3 months after the IFN therapy, 14 (88%) remained clear throughout the observation until 24 months after IFN. They were considered responders to IFN with respect to TTV.

Comparison of Responders and Nonresponders to IFN With Respect to TTV

Table III compares various features between the 14 patients who responded to IFN with respect to TTV and the remaining 17 who did not. No differences were noted in HCV RNA titers, HCV genotypes, or the response to IFN of HCV. Coinfection with GBV-C/HGV seemed to influence the response to IFN of TTV. None of the 14 responders was coinfected with GBV-C/HGV in contrast to 5 of the 17 nonresponders who were coinfected with GBV-C/HGV (0% vs 29%, $P < .05$).

All the four patients with pretreatment TTV DNA titers $\geq 10^3$/ml failed to respond to IFN.

DISCUSSION

There have been several lines of evidence for the association of TTV with hepatitis of unknown etiology [Charlton et al., 1998; Fujiwara et al., 1998; Nishizawa et al., 1998; Okamoto et al., 1998a]. In addition, TTV DNA is detected at levels from 10 to 100 times higher in the liver than in the corresponding serum from some patients with chronic hepatitis of unknown etiology [Okamoto et al., 1998a]. The exact hepatitis-inducing capacity and hepatotropism, as well as replication in the liver, however, need to be evaluated in additional studies.

Should TTV turn out to be a significant cause of hepatitis, therapy in infected patients would be required. Therefore, the sensitivity of TTV to IFN was evaluated in the patients in whom the effect of IFN on HCV and GBV-C/HGV had been studied [Inoue et al., 1997]. When the 96 patients with chronic hepatitis C receiving IFN were tested for TTV DNA, 31 (32%) had positive results at a frequency higher than that of GBV-C/HGV RNA (11%).

Of the 31 patients who were coinfected with HCV and TTV, 14 (45%) responded to IFN with respect to TTV. The response rate, judged by the clearance of viral nucleic acids persisting for at least 6 months after the completion of IFN, was higher than that for HCV

(34%) or GBV-C/HGV (19%) in the same cohort of patients [Inoue et al., 1997]. Hence TTV would be sensitive to IFN therapy. All the four patients with TTV DNA titers $\geq 10^3$/ml failed to clear the infection after IFN. Therefore, pretreatment viral titers may influence the response of TTV to IFN, just as the responses of HCV and GBV-C/HGV [Hino et al., 1994; Inoue et al., 1997].

As new hepatitis-associated viruses are discovered, it has become increasingly evident that the coinfection with multiple viruses is common in the patients with hepatitis. Hepatitis viruses may cooperate for inducing severe liver damage leading eventually to hepatocellular carcinoma [Kaklamani et al., 1991; Benvegnu et al., 1994]. Alternatively, they may interfere with each other and ameliorate liver disease [Pontisso et al., 1993; Cavanaugh et al., 1998].

Coinfection with GBV-C/HGV seems to interfere with the response of TTV to IFN. None of the five patients with a triple infection with HCV, GBV-C/HGV, and TTV responded to IFN with respect to TTV. Moreover, only one in each group responded to IFN with respect to HCV or GBV-C/HGV. Therefore, coinfection with known and as yet unidentified hepatitis viruses would need to be considered in the treatment of chronic hepatitis with IFN.

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